

Evaluation of biodiesels from several oilseed sources as environmental friendly contact herbicides[☆]

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Abstract

Postemergence contact herbicidal activities of biodiesels from several oilseed sources were examined for potential use to control broadleaf weeds in turfgrasses. Cuphea, lesquerella, meadowfoam, milkweed and soybean oils were applied as 1 and 2% (v/v) aqueous emulsions plus a nonionic surfactant to 2-week-old seedlings of perennial ryegrass, velvetleaf and sicklepod in the greenhouse. All five biodiesels were more phytotoxic to sicklepod and velvetleaf than to perennial ryegrass. Several different surfactants were tested together as emulsions with 1% soybean biodiesel, with Triton X-100 being the most effective. These results indicate that biodiesels may be useful as environmental friendly contact herbicides in turfgrasses, as there are few contact herbicides options available for the homeowner market which will not cause turf injury.

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1. Introduction

Petroleum oils have been used to kill weeds, generally as non-selective contact herbicides, since about 1940 (Klingman and Ashton, 1981). These petroleum oils include such hydrocarbons as petroleum distillates, gasoline, kerosene, and diesel fuel. The toxicity of these oils to plants has been correlated with their chemical structure, with shorter-chain hydrocarbons generally having more toxicity than longer-chain compounds (Klingman

and Ashton, 1981). The herbicidal mechanism of action of these petroleum oils has been associated with their ability to break through the cuticle of the target plant and solubilize the lipids of the cell membranes, causing tissue dessication and death. Despite their non-specific activity, when used at proper rates certain petroleum oils, such as kerosene, have been shown to kill dandelions in bluegrass turf without significantly injuring the turf (Loomis, 1938).

Biodiesel is a renewable fuel comprised of the mono-alkyl esters of fatty acids primarily derived from vegetable oils (Schwab et al., 1987; Van Gerpen, 2005). Biodiesel is typically produced by reacting vegetable oils with an alcohol such as methanol or ethanol in the presence of a catalyst to yield the mono-alkyl esters and glycerin. Mono-alkyl esters of eight, 10 and 12 carbon fatty acids have been found to be effective chemical

[☆] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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pruning agents for the prevention of side shoot growth for several plants, including tobacco and tomato (Cathey et al., 1966; Tucker and Maw, 1975; Logendra et al., 2001). Methylated vegetable oils, which consist of the methyl esters of fatty acids, have also been found to be effective adjuvants for several postemergence herbicides (Nalewaja et al., 1995). Esters of fatty acids work in a similar manner as petroleum oils by disrupting plant cell membranes in surface cells, leading to tissue death.

Unlike petroleum oils which are moderately toxic and are slow to degrade in the environment, biodiesel has very low mammalian toxicity with LD₅₀ values greater than 5000 mg/kg when administered orally to rats and is rapidly degraded in soil and water by microorganisms (Zhang et al., 1998; Peterson and Möller, 2005). Other naturally occurring compounds that have been marketed as organic contact herbicides include vinegar (acetic acid), pelargonic (nonanoic) acid and essential oils such as clove and wintergreen (Duke et al., 2002; Twokorski, 2002; Bainard et al., 2006). However, vinegar and pelargonic are irritants to mucus membranes while wintergreen oil (~98% methyl salicylate) is also fairly toxic (ingestion of as little as 4 mL can be lethal to children) (Leung, 1980). Additionally, commercially available biodiesel is relatively inexpensive, currently selling for about US\$ 1 kg⁻¹, while several of these naturally occurring compounds are quite expensive (Anon., 2006). In this study we report the postemergence herbicidal activity of biodiesels produced from several different oil sources towards a turfgrass species, perennial ryegrass (*Lolium perenne* L.) and two broadleaf weed species, sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] and velvetleaf (*Abutilon theophrasti* Medik.), and also report the activity of soybean biodiesel when applied together with several different non-ionic and ionic surfactants.

2. Materials and methods

2.1. Materials

Perennial ryegrass ('Federation') seed was obtained from Kelly Seed and Hardware (Peoria, IL). Velvetleaf seed was obtained from Valley Seed Service (Fresno, CA). Sicklepod seed was obtained from Dr. Rogers Harry-O'kuru, NCAUR, Peoria, IL. Milkweed (a mixture of *Asclepias speciosa* Torr. and *A. syriaca* L.) seed oils were obtained from seeds provided by the Natural Fiber Corp. (Ogallala, NE). Cuphea (*C. viscosissima* x *C. lanceolata* f. *silenoides*) and lesquerella (*Lesquerella fendleri* L.) oils were obtained from Drs. Terry Isbell and Stephen Cermak, NCAUR. Meadowfoam (*Limnan-*

thes alba Hartw.) oil was obtained from Natural Plant Products, Inc. (Salem, OR). Refined soybean oil was obtained from Bunge North America, Inc. (Decatur, IN). Milkweed seeds were obtained from Natural Fiber Corp. (Ogallala, NE) and the crude press oil was refined as described previously (Holser and Harry-O'kuru, 2006). Commercial soybean methyl esters were obtained from Ag Environmental Products, LLC (Omaha, NE).

2.1.1. Preparation of esters

Vegetable oils were transesterified in 20-g batches with excess methanol at a 1:6 molar ratio and 2 wt% methoxide catalyst to produce the methyl esters. Reactions were performed in 50-mL glass flasks. Reactants were placed into the flask with a magnetic stir bar. Sodium methoxide catalyst was dissolved in methanol and then added to the vegetable oil. This mixture was heated to 60 °C on a hotplate and magnetically stirred at 500 rpm. After 1-h the mixture was removed from the flask and the product was separated from the lower aqueous glycerol layer by gravity. The esters were washed 2× with distilled water. The washed esters were dried under vacuum in a rotary evaporator and stored at 5 °C.

2.1.2. Preparation of soybean oil glycerides (SBOG)

A 20-g quantity of refined soybean oil was placed in a 50 mL glass flask with 5 g glycerol and 30 mg LiOH catalyst. A small magnetic stir bar was added and the flask was placed on a hotplate and heated to 190 °C with stirring. After 30 min the reaction products were removed, cooled, and analyzed. Analysis by GC-FID indicated a composition of 72% monoglycerides, 10% diglycerides, and 18% triglycerides in the mixture. The same composition was obtained when the reaction time was increased to 90 min.

2.2. Gas chromatography-flame ionization detector (GC-FID)

Samples were analyzed with an Agilent 6890 gas chromatograph using a DB-5HT column measuring 15 m × 0.32 mm × 0.1 micron film thickness (J&W Scientific, Folsom, CA). The carrier gas was Helium flowing at 5 mL/min. The oven was programmed from an initial temperature of 50–180 °C at 15 °C/min, increased to 230 at 7°/min, and then to 380 °C at 30 °C/min with a 10 min hold for a total run time of 31 min. Injection volumes were 1 µL with the inlet set to splitless mode. Detection of eluents was made by FID and identified by retention times compared to known standards. Glycerol, fatty acid esters, monoacylglycerides, diacylglycerides, and tria-

Table 1
Percentage fatty acid profile of biodiesels

Fatty acids	Cuphea	Lesquerella	Meadowfoam	Milkweed	Soybean
Saturated					
C8:0	0.5	–	–	–	–
C10:0	70.1	–	–	–	–
C12:0	2.0	–	–	–	–
C14:0	4.5	–	–	–	–
C16:0	6.4	1.0	–	5.9	12.9
C18:0	0.7	1.8	0.5	2.3	3.7
C20:0	–	–	–	0.2	–
Unsaturated					
C16:1	–	–	–	6.8	0.1
C18:1	9.1	17.9	1.4	34.8	22.2
C18:2	6.7	6.5	0.5	48.7	52.9
C18:3	–	9.7	–	1.2	7.9
C20:1	–	–	65.0	–	–
C20:1-OH	–	54.7	–	–	–
C22:1	–	–	13.2	–	–
C22:1-OH	–	3.0	–	–	–
C22:2	–	–	19.3	–	–

cylglyceride standards were used, and fatty acid profiles (Table 1) were determined by FID of these standards. Data were collected and processed via Chemstation software (Agilent Technologies, Inc., Palo Alto, CA).

2.3. Bioassays

Perennial ryegrass, sicklepod and velvetleaf seeds (100, 20 and 20 seeds, respectively) were planted in 155-mm pots containing Redi-Earth (79 L; Sun Gro Horticulture, Bellevue, WA) supplemented with 4 L perlite (Lite Weight Products, Inc., Kansas City, KS), 400 g Osmocote 14-14-14 and 90 g Micromax micronutrient fertilizer (Scotts, Marysville, OH). After emergence sicklepod and velvetleaf were thinned to 10 plants/pot. Plants were grown for 2 weeks at which time 1 and 2% (v/v) biodiesel emulsions formulated with 0.5% (v/v) Triton X-100 (Sigma, St. Louis, MO), as a surfactant were applied using a spray bottle to all aboveground areas of the plants (application rate 50 mL m^{-2} ; controls consisted of Triton X-100 only. This rate of Triton X-100 was chosen as it was the lowest level that produced stable emulsions). Emulsions were prepared by adding biodiesels and surfactants together neat, after which water was added and the solution was vigorously agitated for approximately 20 s, which produced a milky emulsion which would not separate during application. Plants were assayed after 72 h for damage on a scale from 1 to 5, with 1 being no visible damage to 5 being complete tissue necrosis. Plants were excised at soil level and fresh weights were taken. Soybean

biodiesel, the only commercially available biodiesel used in this study, was subsequently tested at the 1% rate together with several surfactants, also at the 1% rate. Surfactants tested were SBOG, Tween-20 (Bio-Rad Laboratories, Hercules CA), sodium dodecyl sulfate (SDS; Sigma), Herbicide Helper™ (HH; Lawn and Garden Products, Inc., Fresno, CA), and Triton X-100. SBOG, Tween-20 and Triton X-100 are non-ionic surfactants, HH is a commercial mixture of petroleum distillates and alkylphenoxypolyethoxy ethanols, while SDS is an ionic surfactant. All experiments had three replicates and were repeated. Data from both experiments were pooled because there was no significant experiment-by-experiment interaction, and subjected to analysis of variance. All statistical analyses were performed using JMP 3.1.6 (SAS Institute, Inc., Cary, NC, USA). Means were separated by Fisher's Protected LSD at the 5% probability.

3. Results and discussion

3.1. General

Fatty acid profiles of the biodiesels tested in this study are shown in Table 1. Soybean oil was chosen because it is one of the oils used commercially to produce biodiesel, while the other oils were chosen because of their varied fatty acid profiles. Soybean oil is comprised principally of linoleic and linolenic acids with low levels ($\sim 16\%$) of saturates. Milkweed oil is similar to soybean having even lower ($\sim 8\%$) saturates.

Meadowfoam oil is unique in that over 95% of its acyl groups are longer than C18, about 90% of these fatty acids have double bonds in the Δ_5 position, and the oil contains very little polyunsaturates. Lesquerella oil is rich in hydroxy fatty acids, containing over 50% lesquerolic acid (14-hydroxy-*cis*-11-octadecenoic acid). Cuphea oil has medium chain fatty acids, being comprised of over 80% capric (decanoic) acid.

3.2. Herbicidal activity of biodiesels

Perennial ryegrass was chosen as the test species in our experiments rather than other turfgrass species such as Kentucky bluegrass (*Poa pratensis* L.) due to rapid seed germination and plant growth which was similar to that of sicklepod and velvetleaf. The biodiesel rates used in this study were determined by preliminary bioassays using the same test species with soybean biodiesel at rates of 0.1%, 0.25%, 0.5%, 1.0%, 2.0%, 5.0%, and 10%. This showed that rates less than 1% were not sufficiently phytotoxic to the two weed species while the 5% and 10% rates caused unacceptable injury to the perennial ryegrass (results not shown). In general the biodiesels were more phytotoxic at the 2% application rate (Table 2). As previously mentioned, in preliminary tests we examined biodiesels at rates up to 10%, but at the higher rates tested (5 and 10%) severe injury to perennial ryegrass plants occurred, so only concentrations of 1 and 2% were used in this study. Triton X-100 alone caused some slight damage to leaf tissues of sicklepod and velvetleaf, but no noticeable injury to perennial ryegrass. At the 1% rate none of the biodiesels tested caused complete tissue necrosis (a damage rating of 5), which would cause the death of the plant. Cuphea, meadowfoam, and milkweed biodiesels were particularly active against sicklepod and velvetleaf, having damage ratings at or near the maximum and greatly reducing fresh weight. Only the 2% cuphea biodiesel caused significant injury to the perennial ryegrass; this is important as the use of these compounds in turf necessitates that they cause little or no injury to the turf while being effective against the target weeds. Sicklepod and velvetleaf plants showed signs of injury within 1 h of treatment, and by 72 h maximum injury occurred. This rapid tissue necrosis also occurs with pelargonic acid and with essential oils such as thyme, cinnamon and clove (Twokorski, 2002). As previously discussed, petroleum-based diesel oils have been used as contact herbicides for total vegetation control. However, they were generally found to be too phytotoxic for selective weed control, such as controlling broadleaf weeds in turfgrasses (Klingman and Ashton, 1981).

Table 2

Activity of biodiesels as postemergence herbicides applied as aqueous emulsions with 1% Triton X-100

Bioassay species	Damage rating ^a	Fresh weight (% of control) ^b
Perennial ryegrass		
1% cuphea BD	1.2a	82.3b
2% cuphea BD	3.0c	46.1d
1% lesquerella BD	1.0a	103.0a
2% lesquerella BD	1.0a	86.7b
1% meadowfoam BD	1.0a	99.2a
2% meadowfoam BD	1.5b	63.2c
1% milkweed BD	1.1a	68.5c
2% milkweed BD	1.7b c	60.4c
1% soybean BD	1.8b c	86.6b
2% soybean BD	1.0a	71.0b c
Sicklepod		
1% cuphea BD	3.5b	60.8b c
2% cuphea BD	4.8c	45.4c
1% lesquerella BD	2.7a	81.3a
2% lesquerella BD	3.8b	63.8b c
1% meadowfoam BD	3.1a b	69.7b
2% meadowfoam BD	5.0c	43.1c
1% milkweed BD	3.5b	49.5c
2% milkweed BD	4.7c	48.8c
1% soybean BD	3.8b	72.9b
2% soybean BD	4.0b	55.7b c
Velvetleaf		
1% cuphea BD	3.0b	32.8b
2% cuphea BD	3.3b	27.6c
1% lesquerella BD	1.8a	44.9a
2% lesquerella BD	3.0b	43.9a
1% meadowfoam BD	2.8b	28.6b c
2% meadowfoam BD	5.0d	19.7d
1% milkweed BD	3.1b	36.4b
2% milkweed BD	5.0d	18.4d
1% soybean BD	3.9c	21.5c d
2% soybean BD	4.2c	24.7c

^a Plants were assayed after 72 h for damage on a scale from 1 to 5, with 1 being no visible damage to 5 being complete tissue necrosis. Means followed by the same letter within a species and column are not significantly different at the $p \leq 0.05$ level.

^b Controls consisted of Triton X-100 only.

Plants sprayed with biodiesels exhibited similar effects to those found with petroleum oils. Affected plants initially showed a darkening of the younger leaves, giving the treated plants a water-soaked appearance. After 1–2 h there was a loss of turgidity and drooping of leaves and stems. Biodiesel-treated plants, especially sicklepod, had the odor of freshly cut grass, indicating the production of lipooxygenase metabolites such as *cis*-3-hexenal and *trans*-2-hexenal (Gardner, 1995).

Herbicidal activity of soybean biodiesel was greatly influenced by surfactants and varied between species (Table 3). Triton X-100, which was used in the evalu-

Table 3

Activity of 1% soybean biodiesel (SBBD) as a postemergence herbicide when formulated as emulsions with various surfactants

Bioassay species	Damage rating ^a	Fresh weight (% of control) ^b
Perennial ryegrass		
Triton X-100	1.8c	73.4c
Triton X-100 + SBBD	2.1c	67.5c
Tween 20	1.0a	101.8a
Tween 20 + SBBD	1.0a	90.1b
SBOG	1.0a	101.2a
SBOG + SBBD	1.0a	91.8b
SDS	1.0a	95.8a b
SDS + SBBD	1.0a	100.0a
HH	1.0a	101.3a
HH + SBBD	1.4b	76.1c
Sicklepod		
Triton X-100	3.0c	35.8d
Triton X-100 + SBBD	4.8d	27.4d
Tween 20	2.0b	92.7a
Tween 20 + SBBD	2.2b	84.3b
SBOG	1.0a	94.6a
SBOG + SBBD	2.0b	76.3b c
SDS	2.0b	75.2b c
SDS + SBBD	3.0c	57.2c
HH	1.8b	83.1b
HH + SBBD	2.0b	77.4b c
Velvetleaf		
Triton X-100	2.0c	68.2c
Triton X-100 + SBBD	4.7d	26.0d
Tween 20	1.0a	99.0a
Tween 20 + SBBD	1.3b	91.5b
SBOG	1.0a	100.4a
SBOG + SBBD	2.3c	74.9c
SDS	1.0a	101.4a
SDS + SBBD	1.3b	93.9a b
HH	1.0a	105.5a
HH + SBBD	1.0a	92.0b

^a Plants were assayed after 72 h for damage on a scale from 1 to 5, with 1 being no visible damage to 5 being complete tissue necrosis. Means followed by the same letter within a species and column are not significantly different at the $p \leq 0.05$ level.

^b Control consisted of water without surfactants.

ation of the different biodiesels at a lower concentration (0.5%), was the most effective in enhancing herbicidal activity, but caused some damage to perennial ryegrass plants by itself, and caused significant damage to both sicklepod and velvetleaf. None of the other surfactants damaged perennial ryegrass or velvetleaf, but Tween 20, SBOG and HH all were injurious to sicklepod. When used with Triton X-100 the soybean biodiesel caused extensive injury to sicklepod and velvetleaf, which was sufficiently severe enough to result in plant death. In several cases fresh weights of perennial ryegrass were decreased even though the plants appeared undamaged.

Surfactants have been shown to differ greatly in effectiveness with various herbicides (Manthey et al., 1992; Green and Green, 1993; Nalewaja et al., 1995). Much of the herbicidal activity caused by the biodiesels in this study depended upon the surfactant used. This was also found by Cathey et al. (1966) when examining the action of mono-alkyl esters of fatty acids (similar to the biodiesels) on plant meristems. Generally non-ionic rather than ionic surfactants are recommended by agrichemical companies as surfactants to be used in conjunction with herbicides (Nalewaja et al., 1995). In our study the most effective surfactant was nonionic (Triton X-100) while the ionic surfactant tested (SDS) was less effective.

Other “natural” herbicides such as vinegar (primarily acetic acid) and pelargonic (nonanoic) acid have been found to possess greater phytotoxicity to broadleaf plants than grasses (Webber and Shrefler, 2006a,b). In both of these studies the active compounds were applied at rates up to 10% (household vinegar is typically 5%), and activity increased with the use of surfactants. Both compounds are contact herbicides causing rapid visual injury to treated plant tissues, in a similar manner to biodiesel-treated plants.

Because biodiesels are chemically synthesized, they would not qualify as “organic” herbicides under the current USDA National Organic Program Standards (NOPS), which allows compounds such as vinegar (from fermentation processes) and clove oil (extracted by synthetic chemical solvents from plant materials) to be used (U.S. Department of Agriculture, 2000). However, a limited number of synthesized substances are allowed under the NOPS, and it is possible that in the future biodiesels could be granted use in organic production systems. In either case, this would not prevent these compounds from being utilized by homeowners or in other situations where environmental concerns are paramount.

4. Conclusions

The results of this study suggest that biodiesels in conjunction with certain surfactants can act as contact herbicides to kill broadleaf weeds in turfgrass. The effective rates that we found are as low or lower than other organic or “natural” compounds used as herbicides. Although the biodiesels would not currently qualify as organic herbicides by standards of the USDA’s National Organic Program, the low economic costs of the biodiesels combined with their low environmental impact and low risk of over-application should help make them attractive to consumers. Further field studies con-

cerning their use to control a wider variety of weeds in other turfgrass species are warranted.

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